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Sustainable Phosphorus and Protein Recovery from Different Organic Wastes: Process Optimization and Struvite Precipitation Potential

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Abstract

Currently, researchers are exploring alternative phosphorus sources for agricultural production that are more sustainable than rock phosphate. In this context, the recovery of phosphorus from organic wastes as struvite can constitute an important tool for promoting circular economy practices and reducing the risk of phosphorus contamination through eutrophication. Struvite recovery has been widely developed using different organic wastes with high concentrations of N and P, such as industrial, municipal and animal wastes, mainly in the form of effluents. However, little information is available concerning phosphorus recovery in the form of struvite from sewage sludge samples, these processes being mainly based on chemical procedures. Therefore, the main aim of this work was to study phosphorus recovery from three sewage sludge samples from different wastewater treatment plants (SS1, SS2 and SS3), in comparison with the solid fraction of pig manure (M), through an optimized bioacidification process, as well as to evaluate the potential for struvite precipitation from the recovered P-rich supernatants. Protein recovery through alkaline treatment of the remaining precipitates was also studied. The results obtained showed the feasibility of the optimized bioacidification process for P recovery, especially in the samples M and SS3, which showed the highest P recovery yields (65.7% and 69%, respectively) and the best results regarding struvite formation. In addition, the protein recovery efficiency of the remaining solid residues ranged from 59.3% to 67.4%, without showing a clear influence of the type of organic waste used.

Keywords: bioacidification; FESEM-EDX; phosphorus extraction; pig slurry; sewage sludge; struvite; XRD

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1. Introduction

Phosphorus is an essential nutrient in plant nutrition. The availability of phosphorus poses a challenge for food production maintenance in a global context of population growth.

The P fertilizer industry relies mostly on P_2O_5 rock (PR), which is a non-renewable resource generally existing in old marine deposits, with the associated physical, economic, energy or legal constraints [1]. PR reserves are concentrated in a few countries. Morocco alone holds 77% of global reserves, estimated at 50,000 tons [2]. PR is manufactured into inorganic orthophosphate-based fertilizers, such as monoammonium phosphate, diammonium phosphate and triple superphosphate [3]. Traditional inorganic P fertilizers use is associated with inefficient P use in soils, with the majority of this nutrient not being used by plants and being immobilized into soils or leached towards water bodies [4]. P is irregularly distributed in soil, with surpluses around extensive farms and urban areas [5].

Therefore, it is crucial to find alternative phosphorus sources to achieve independence from geopolitical factors and to improve phosphorus use efficiency by crops to ensure global food production. The direct application of compost or stabilized sludge, manure, digestate from anaerobic digestion or sludge ashes is a widespread strategy that is being limited due to concerns about P leaks and heavy metals, antibiotic residues and pathogen contamination [6]. Thus, further processing of these residues to obtain environmentally friendly, specific fertilizers is desirable. Recovery of nutrients such as P from these residues is important to promote circular economy practices and reduce the risk of P pollution through eutrophication. In this sense, the current most popular recovered P mineral from organic wastes is struvite ($MgNH_4PO_4{\cdot}6\ H_2O$), a P mineral formed from magnesium (Mg^{2+}), ammonium (NH^{4+}) and orthophosphate ($PO_4{}^{3-}$) at a molar ratio of 1:1:1. Due to its composition (approximately 10% magnesium (Mg), 7% ammonium (N), 39% phosphate (P) and 44% crystal water by mass [7], this mineral has gained significant interest in recent years as potential substitute for inorganic fertilizers based on PR, which also implies sustainable practices in nitrogen and phosphorus recycling [8]. Furthermore, organic production systems, usually based on organic fertilizers such as manures or composts, demand high-grade natural fertilizers to increase their productivity [9]. In this sense, 'recovered struvite and precipitated phosphate salts' have been approved for use in organic production in the European Union by their inclusion in annex II of Regulation 2021/1165 [10].

The recovery of phosphorus as struvite has been assessed in different organic wastes with high concentrations of N and P, such as industrial, municipal and animal wastes [11] (Table S1). Thus, the recovery of struvite has been studied in the wastewaters from different industries, such as slaughterhouse [12], food processing [13] and even textile [14], among others [11]. In the case of animal wastes, the recovery of P as struvite has been mainly reported in swine manures [15–19] and their anaerobic digestates [20,21], as well as in dairy manures [22,23]. However, municipal wastes, mainly wastewaters, have been one of the main feedstock sources studied for struvite recovery, due to the spontaneous precipitation of this mineral in the municipal wastewater treatment plants [11]. Thus, struvite recovery is mainly developed in the effluents from anaerobic sludge digestion of municipal wastewater, since in other wastes, such as municipal wastewater, landfill leachate, human urine and ash from sewage sludge incineration, chemical supplementation or/and pre-treatments are required due to their characteristics [11]. However, studies on phosphorus recovery in the form of struvite from sewage sludge samples are scarce, with most recovery processes being mainly based on chemical processes, such as the wet oxidation [24–26].

In this context, since the main purpose is to recover phosphorus, the first step is to dissolve the inorganic solids present in the organic waste stream used as P source, which contain most of the phosphorus, usually by chemical or biological acidification [27]. Thus, the process of recovering phosphorus as struvite can be mainly based on the following steps: acidic dissolution, solid–liquid separation and pH increase in the liquid phase using magnesium oxide (MgO) or magnesium hydroxide ($Mg(OH)_2$), leading to phosphorus precipitation [28]. Different chemicals have been used to dissolve P, such as

hydrochloric acid [19], formate [29] and citrate [30]. However, the acidification process using chemicals has a significant economic cost and negative relative impacts on the environment [29,31,32]. In this context, a new process alternative to chemical acidification is the biological acidification process or bioacidification. Bioacidification constitutes an innovative and environmentally friendly process in which a medium is acidified by the activity of native or added microorganisms [33,34], usually by a fermentative metabolism in anaerobic conditions [35]. To induce the process, the incorporation of easily biodegradable organic substrates such as sugar [35,36] and food waste has been reported [37]. However, very little information is available regarding the use of this process for phosphorus dissolution to facilitate its recovery from organic wastes, having been reported only recently in sewage sludge [35,36] and pig slurry [38].

After phosphorus dissolution, struvite precipitation can usually be developed by adding different salts, such as $MgSO_4$, $MgCl_2$, $Mg(OH)_2$ and MgO [39]. Generally, struvite precipitation is carried out using high concentrations of $MgCl_2$, followed by base application (e.g., NaOH or KOH) or adding Mg in the form of MgO or $Mg(OH)_2$ and allowing the pH to increase naturally [40]. According to previous studies [41,42], the molar ratio of magnesium to phosphate must be adjusted to 1.2:1 for optimal precipitation. At low Mg concentrations, a mixture of struvite and hydroxyapatite results from the process [43]. However, one problem that remains unresolved is the processing of the remaining solids once the P extraction is completed. The principal components in wastewater sludge flocs are polysaccharides and proteins [44]. One possible processing approach is alkaline treatment to recover proteins and other macromolecules of industrial interest. Protein recovery by alkaline treatment with sodium hydroxide (NaOH) has already been implemented in carbohydrate-digested rice.

Therefore, the aims of this study were the following: (i) to accomplish P recovery from organic wastes (solid fraction of pig manure and sewage sludge) through lactic fermentation using *Lactobacillus acidophilus*; (ii) to study the potential of struvite precipitation from the recovered P-rich supernatants; and (iii) to recover proteins through alkaline treatment of the remaining solid residues.

2. Materials and Methods

2.1. Organic Sources Used

The sewage sludge (SS) samples used in this experiment (SS1, SS2 and SS3) were obtained from the wastewater treatment plants located at the municipalities of Almazora, Castellón and Nules (Castellón, Spain), respectively. SS1 and SS2 were produced using an activated sludge treatment and then stabilized by anaerobic digestion, while SS3 was produced through the aerobic treatment of the wastewater samples, followed by stabilization in aerobic conditions. All the sludge samples were dehydrated by centrifugation. The solid fraction of pig slurry (M) was considered as control treatment to compare the results obtained with the sewage sludge samples and it was collected from a fattening pig farm placed at Todolella (Castellón, Spain) after a solid–liquid separation using a screw press. A complete characterization of these organic materials is shown in Table 1.

Table 1. Main characteristics of the organic wastes used in the experiment. Data values expressed on a dry matter basis.

	M	**SS1**	**SS2**	**SS3**
Dry weight (%)	27.6 ± 0.3	19.8 ± 5.9	22.2 ± 0.1	24.7 ± 0.3
pH	8.9 ± 0.4	6.2 ± 0.3	7.9 ± 0.0	7.2 ± 0.2
EC (dS m^{-1})	2.3 ± 0.0	1.0 ± 0.2	1.7 ± 0.4	1.4 ± 0.7

Table 1. *Cont.*

	M	SS1	SS2	SS3
TC (%)	29.6 ± 0.4	32.7 ± 0.5	30.0 ± 0.4	38.4 ± 0.3
TN (%)	2.3 ± 0.8	6.1 ± 0.2	5.1 ± 0.2	7.1 ± 0.0
NH_4 (g kg^{-1})	0.9 ± 0.1	2.3 ± 0.7	4.0 ± 0.7	2.1 ± 0.5
NO_3 (mg kg^{-1})	185 ± 35	599 ± 20	175 ± 21	109 ± 16
P (%)	3.9 ± 0.2	3.3 ± 0.4	3.3 ± 0.5	2.5 ± 0.5
K (%)	1.6 ± 0.5	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.2
Ca (%)	5.9 ± 0.1	4.7 ± 1.7	6.1 ± 0.3	3.2 ± 0.3
Mg (%)	3.1 ± 0.8	0.7 ± 0.3	0.7 ± 0.1	0.8 ± 0.3
Na (%)	0.5 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
Fe (g kg^{-1})	3.67 ± 0.01	18.4 ± 0.1	45.7 ± 0.2	4.65 ± 0.01
Cu (mg kg^{-1})	317 ± 1	172 ± 2	222 ± 0	183 ± 0
Mn (mg kg^{-1})	1198 ± 1	108 ± 0	177 ± 1	59 ± 2
Zn (mg kg^{-1})	1355 ± 0	2209 ± 0	1006 ± 1	1206 ± 0
Cd (mg kg^{-1})	0.31 ± 0.04	2.71 ± 0.80	1.35 ± 0.38	1.00 ± 0.25
Cr (mg kg^{-1})	13.2 ± 2.9	196 ± 2	97.3 ± 3.5	33.9 ± 2.1
Hg (mg kg^{-1})	0.66 ± 0.02	2.99 ± 0.03	1.81 ± 0.78	0.94 ± 0.31
Ni (mg kg^{-1})	19.9 ± 1.5	118 ± 2	145 ± 5	35.8 ± 0.3
Pb (mg kg^{-1})	< 0.01 ± 0.00	67.9 ± 1.7	156 ± 0	42.7 ± 1.2

M: solid fraction of pig slurry; SS1, SS2 and SS3: sewage sludge samples from wastewater municipal treatments plants of the municipalities of Almazora, Castellón and Nules, respectively. EC: electrical conductivity; TC: total organic carbon: TN: total nitrogen. Data values are reported as mean value ± standard deviation.

2.2. General Procedure for Phosphorus Extraction Through Fermentation

The general methodology used for the phosphorus acid extraction and protein recovery through fermentation was based on a modified version of the procedure of Vanotti and Szogi [45]. Briefly, the biological materials, such as organic wastes, are dissolved in an acidic solution; in this study, the solution was developed by fermentation. The resulting supernatant from the acidic solution (acidic supernatant) may then be separated and used for phosphorus recovery. The resulting precipitate from the acidic treatment (acidic precipitate) may be separated from the supernatant and treated with an alkaline solution, obtaining a supernatant (alkaline supernatant) that can be separated and used to extract proteins. In this study, the phosphorus acid extraction was carried out trying to optimize the fermentation process to obtain the acid precursor solution. For this, a *L. acidophilus* suspension was studied, along with different factors, such as the sugar source, the sugar-to-waste ratio and the incubation time for the fermentation.

Lactobacillus acidophilus suspension was prepared by dissolving 1.1 g *L. acidophilus* (Lacto10, *L. acidophilus* SGL11 strain, Forza Vitale Italia srl., Corato, Italy) in 25 mL of deionized water. *L. acidophilus* was used in lyophilized form at a dose equivalent to 10^8 UFC/mL. The mixture was centrifuged at 3600 rpm for 10 min, and the supernatant was discarded. Then, 20 mL of water were added to the remaining solid to obtain the *L. acidophilus* suspension.

The general procedure for the acid extraction of phosphorus through fermentation in all the assays was the following: an acid precursor solution based on the mixture of an optimized weight of a sugar source and the *L. acidophilus* suspension was mixed with the corresponding organic waste (2 g dry basis) in a 10:1 ratio (volume/weight) of precursor to residue. The different mixtures (depending on the organic waste used) were incubated in a thermostatic bath at a constant temperature (37 °C), in darkness and under continuous agitation (shaking frequency of 35 rpm) for an optimized incubation time, monitoring the pH decrease during the fermentative process. Afterward, the mixtures were centrifuged at 3600 rpm for 30 min to obtain the acidic supernatant and a first precipitate (acidic precipitate,

pp1). One rinse of this precipitate was performed by the addition of an equivalent volume (20 mL) of water and centrifuged under the same conditions, obtaining a supernatant and a second precipitate (pp2). Afterward, the phosphorus concentration was determined in the acidic supernatant and in the supernatant resulting of the precipitate rinse, being expressed as the sum of both contributions. The resulting precipitate from the rinse procedure (pp2) was pasteurized at 70 °C for 1 h and used for the alkaline protein extraction.

2.3. Optimization of Phosphorus Extraction Through Fermentation: Sugar Source, Ratio Sugar/Waste and Incubation Time

The fermentation to extract phosphorus from each selected type of organic waste was optimized using the general procedure previously detailed, but considering two different factors: (a) the sugar source and sugar-to-waste ratio; (b) duration of the fermentation process.

2.3.1. Optimization of the Sugar Source and Sugar-to-Waste Ratio

Two sugar sources, sucrose (Condalab, S.A., Madrid, Spain) and molasse from sugar cane (Poballe, S.A., Barcelona, Spain), were used in the fermentation process for phosphorus extraction. Different amounts (0.25, 0.5, 1, 2 and 3 g) of each type of sugar were added to the fermentation media, respectively. A control assay with no sugar addition was also established.

2.3.2. Incubation Assay to Optimize the Incubation Time

Different incubation times were applied (0 h, 6 h, 12 h, 24 h, 36 h and 48 h), monitoring the pH during the incubation process and considering that fermentation had been completed when pH was stabilized.

2.4. Alkaline Protein Extraction

The precipitate resulting from the rinse procedure (pp2) was mixed with a 0.4 M NaOH solution (pellets for analysis, ACS, ISO, ITW Reagents Panreac, Barcelona, Spain) with stirring for 20 min, followed by homogenization with ULTRA-TURRAX for 10 min and centrifugation at 3600 rpm for 30 min to obtain an alkaline supernatant and an alkaline precipitate (ap). This precipitate was rinsed with 20 mL of water and centrifuged under the same conditions, obtaining a second supernatant (rinse supernatant). The total protein extracted was determined in both supernatants (alkaline and rinse supernatant) using the Bradford assay [46] and expressed as the sum of both contributions.

2.5. Analytical Characterization of the Organic Wastes and Precipitates

The pH and electrical conductivity (EC) of the organic wastes were determined in a 1:10 (w/v) water extract, while nitrate (NO_3^-) and ammonium (NH_4^+) were measured in a 1:5 (w/v) extract with 0.2 M KCl using a multiparametric analyzer K-365 Dist Line (Büchi Labortechnik AG, Flawil, Swiss). The rest of the parameters in all the samples (organic wastes and recovered P precipitates, pp1 and pp2) were analyzed. The total C and N contents were determined using an automatic elemental microanalyzer (EuroVector, Milan, Italy). In the acid extract obtained after microwave digestion with HNO_3 69% (reagent for analysis, ACS, ISO, ITW Reagents Panreac, Spain), P was assessed colorimetrically as molybdovanadate phosphoric acid; K and Na were determined by flame photometry (Jenway PFP7 Flame Photometer, Jenway Ltd., Felsted, Dunmow, Essex, UK); and Ca, Mg, Fe, Mn, Cd, Cr, Cu, Pb, Zn, Ni and Hg were determined by induced coupled plasma mass spectroscopy (ICP-MS), following the methods described by Morales et al. [47].

2.6. Struvite Precipitation and Mineralogical Characterization

The acidic supernatants obtained were used for P precipitation as struvite ($(NH_4)MgPO_4 \cdot 6\ H_2O$), according to Vanotti and Szogi [45]. Briefly, $MgCl_2$ was used as the Mg source due to its high solubility, which favors the formation of a precipitate with a high purity degree. The reagent was added at a dose to maintain a molar Mg:P ratio of 1.2:1. Alkalinization with a solution of NaOH 4 M was also needed to reach a pH of 9. The acidic supernatants collected from the P extraction phase were placed in a beaker under continuous stirring. The 4M NaOH solution was added drop by drop and pH was monitored throughout the whole process. Precipitates were separated from the liquid phase using microfiber filters and a vacuum system, and they were dried in an oven at a temperature below 60 °C for 24 h.

The morphological structure of these precipitates was analyzed using X-ray diffraction analyses, carried out using a Bruker D8-Advance with a Goebel mirror (non-planar samples) and a high temperature chamber (up to 900 °C), equipped with a KRISTALLOFLEX K 760-80F x-ray generator (power: 3000 W, voltage: 20–60 KV and current: 5–80 mA) and a copper-anode RX tube (Bruker Corporation, Billerica, MA, USA). In addition, the precipitates were analyzed using a Schottky-type Sigma 300 VP field emission scanning electron microscope (FESEM) with a coupled energy dispersive X-ray system (EDX) to determine their element composition (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). Samples were imaged under 15 kV electron high tension and using a backscattered secondary electron detector. The samples were covered with a Cr layer of 10 nm. The obtained results were also analyzed through a Rational Mineralogical Analysis [48] to establish the relative proportions of struvite and hydroxyapatite in the samples.

2.7. Statistical Analysis

The statistical analyses conducted to assess the effects of the two factors considered (sugar source and amount) in the fermentation media were conducted using a two-way analysis of variance (ANOVA). The results of the experiment to evaluate the optimal incubation time were analyzed using a repeated-measures analysis of variance within a general linear model. The differences in the phosphorus and protein recovery yields depending on the organic source used were determined using ANOVA. The post hoc analysis was conducted using the Tukey-b test at $p < 0.05$. The statistical analyses were conducted using IBM SPSS Statistics v. 29.0 statistical software package (IBM Corp. Released 2020. Armonk, NY, USA).

3. Results and Discussion

3.1. Optimization of the Phosphorus Extraction: Sugar Source and Ratio

The first step of the procedure optimization in the fermentation process was based on the selection of the best sugar source and the optimal ratio. For this, as described in the previous section, two different sugar sources (sucrose and molasses) were added to the fermentation media, in different amounts (0.25, 0.5, 1, 2 and 3 g), with a control treatment without sugar addition, to study the effects of both factors on the pH of the fermentation media and consequently, on P recovery yield (Figure 1).

The different sugar amounts and the type of sugar had a statistically significant effect (two-way analysis of variance, $p < 0.001$) on the pH of all the fermentation media (Figure 1a), showing that both sugars had a clear acidification effect on the media of all the organic wastes used, indicating a low buffer capacity in these wastes. In the case of molasses, increasing the amount of sugar resulted in an almost linear acidification of the fermentation medium for all the organic sources. This behavior differed for sucrose, which did not show linear acidification with increasing sugar amounts. However, in general, sucrose

showed the highest acidification effect compared to molasse, revealing the lowest pH values (two-way analysis of variance, $p < 0.001$) at 2 g of this sugar. This effect on the bioacidification process is similar to that observed by Piveteau et al. [27] in a study of phosphorus recovery as struvite from pig slurry by biological acidification using sucrose as the sugar source. However, P recovery efficiency was significantly influenced by both the type and amount of sugar source used for the precipitate from M (two-way analysis of variance, $p < 0.001$). The highest P recovery yields for M and SS1 were obtained from fermentation media with sucrose. For SS1, recovery efficiency was influenced only by the sugar amount (two-way analysis of variance, $p < 0.001$) (Figure 1b).

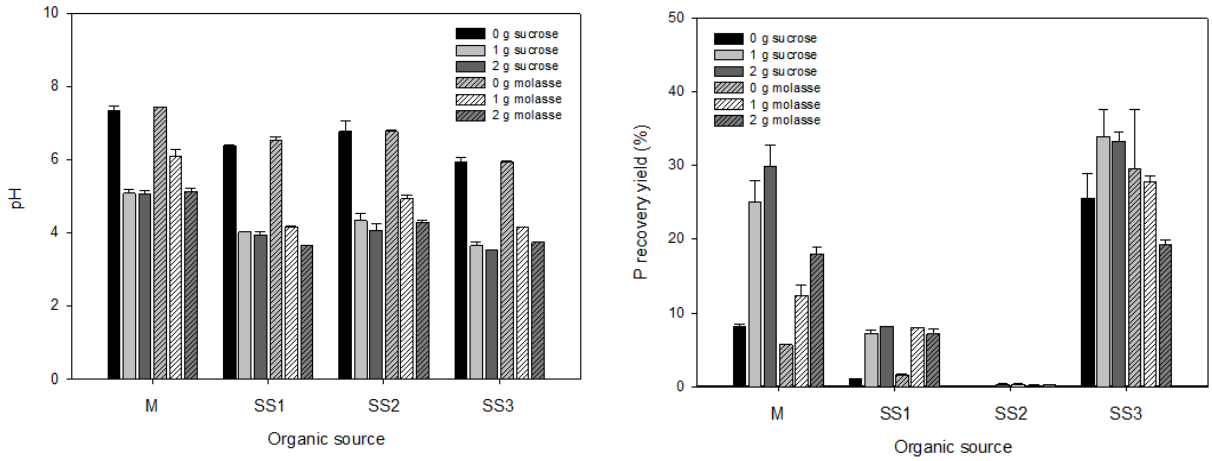


Figure 1. Final pH values in the P recovery extract (**a**) and phosphorus recovery yield (**b**) depending on the amount and type of sugar used for the fermentation process. M: solid fraction of pig slurry; SS1, SS2 and SS3: sewage sludge from wastewater municipal treatments plants in the municipalities of Almazora, Castellón and Nules, respectively. Vertical bars represent standard deviation.

Thus, the amount of sugar seemed to show a significant effect on P recovery for both types of sugars, augmenting the efficiency with increasing amounts of sugar, except for the fermentation media of SS2 and SS3, in the latter even showing the opposite effect when molasses was used as the sugar source. The decrease in P recovery efficiency at higher sucrose concentrations and associated lower pH could be attributed to P assimilation by bacterial biomass, since when pH is close to 5, no additional P is dissolved, and biomass growth can decrease the dissolved P concentration [27].

3.2. Optimization of the Phosphorus Extraction: Incubation Time

The incubation time for the fermentation process was the other factor considered for the optimization of the bioacidification process for phosphorus extraction. For this, the pH values were assessed at different incubation times (0 h, 6 h, 12 h, 24 h, 36 h and 48 h), considering the optimal factors obtained in the first assay (2 g of sucrose) for the fermentation. The process was considered finished when the pH values were stabilized. Table 2 shows the evolution of the pH values during the fermentation process, considering the different incubation times.

The pH values significantly decreased with incubation time in the media of all the organic sources used. The sewage sludge samples (SS1, SS2 and SS3) showed lower initial pH values (repeated measures analysis, $p < 0.001$) compared to the solid fraction of pig slurry (M). After 48 h of incubation, all fermentation media reached their lowest pH values, in the range of 3.58–5.06. Su et al. [49] also reported a stabilization in the phosphorus release rates and the highest phosphate rates at 48 h of fermentation in a study of phosphorus recovery from pig manure using a co-fermentation process with food waste.

Table 2. pH values during the incubation at different times.

Organic Waste	Initial pH	pH After 6 h	pH After 12 h	pH After 24 h	pH After 48 h
M1	8.73	7.51	6.49	5.24	5.06
SS1	6.13	6.00	5.49	4.43	3.97
SS2	7.10	6.32	5.60	4.87	4.13
SS3	5.90	5.38	4.88	4.12	3.58

M: solid fraction of pig slurry; SS1, SS2 and SS3: sewage sludge samples from municipal wastewater treatment plants in the municipalities of Almazora, Castellón and Nules, respectively.

3.3. Phosphorus Extraction Efficiency with the Optimized Procedure

The phosphorus recovery yield and the final pH values for each extract of the different organic wastes used are shown in Figure 2. As can be observed, the extract with SS3 as the organic source showed the lowest pH value (3.58) and consequently, the highest P recovery yield (69.0%) of all the extracts from the sewage sludge samples, which was also reported by Piveteau et al. [27] in an experiment using biological acidification to recover phosphorus as struvite from pig slurry as the organic source. However, this effect was not observed in the rest of the treatments with sewage sludge samples (SS1 and SS2), which showed low pH values and low P recovery yields, or in the treatment with the solid fraction of pig manure (M), which displayed the opposite behavior: a P recovery yield statistically similar to that of SS3 (Figure 2) but with the highest pH value (5.06).

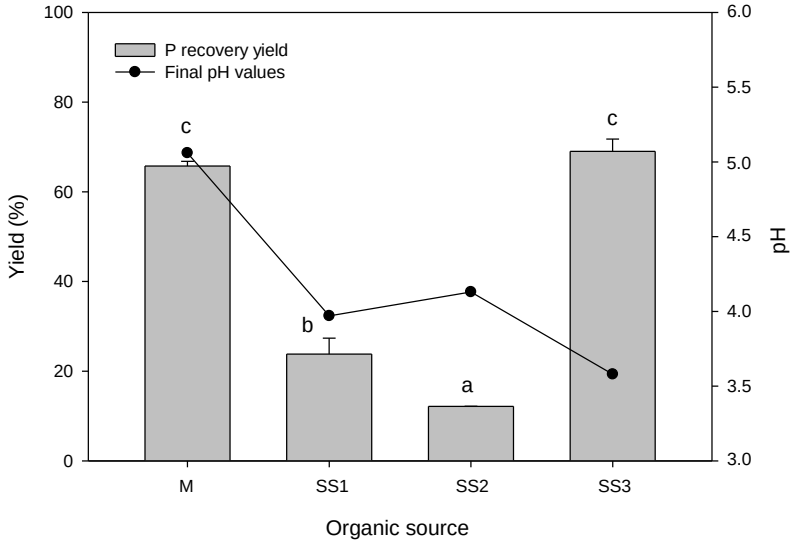


Figure 2. Phosphorus recovery yield with the optimized procedure and final pH values of the extract. Vertical bars represent standard deviation. Mean values with different letters indicate statistical differences between treatments at $p > 0.05$ (Tukey-b test).

The disconnection between dissolved phosphorus and pH could be explained by the processes of hydrolysis of solid organic P and mineralization of dissolved organic matter [27]. Thus, the different nature of the organic sources used and their different contents in organic P, which can constitute up to 22% of total phosphorus [29], could also explain the differences observed. On the other hand, other characteristics of the organic wastes used, such as their iron content, could strongly affect the efficiency of P recovery. Fe has a strong affinity for the P compounds, especially phosphates, forming stable iron–phosphate minerals, which can make P release difficult, depending on the conditions (pH, the presence of organic substances, redox conditions and particle morphology) [50]. Thus, the samples SS1 and SS2 showed the highest Fe concentrations (Table 3), which could explain the low P recovery yields found for these samples (Figure 2).

Table 3. Chemical composition of the recovered precipitates. Data are expressed on a dry matter basis.

	P-M	P-SS1	P-SS2	P-SS3
TC (%)	5.50	19.4	19.0	10.4
TN (%)	4.05	0.44	1.19	2.54
P_2O_5 (%)	17.1	13.2	10.7	16.6
K (%)	1.28	0.38	0.074	0.057
Ca (%)	2.5	10.3	5.3	5.1
Mg (%)	8.97	1.77	0.49	6.28
Na (%)	0.63	2.03	1.31	1.94
Fe (g kg^{-1})	0.73	28.5	17.6	12.0
Cu (mg kg^{-1})	47.0	7.9	20.0	3.6
Mn (mg kg^{-1})	1420	330	884	363
Zn (mg kg^{-1})	490	1079	137	86
Cd (mg kg^{-1})	<0.01	1.64	3.93	<0.01
Cr (mg kg^{-1})	3.76	37.8	108	22.3
Ni (mg kg^{-1})	4.73	103	5.50	7.68
Pb (mg kg^{-1})	2.11	11.6	44.6	6.23

P-M: precipitate from the solid fraction of pig slurry; P-SS1, P-SS2 and P-SS3: precipitates from the sewage sludge samples from municipal wastewater treatment plants in the municipalities of Almazora, Castellón and Nules, respectively. EC: electrical conductivity; TC: total organic carbon: TN: total nitrogen.

3.4. Protein Recovery Efficiency

Regarding the efficiency of protein recovery from the final precipitate (pp2) obtained from the rinse procedure, the values obtained ranged from 59.3%, obtained for the precipitate from SS3, to 67.4% for the precipitate obtained from SS1, showing no significant differences depending on the organic source (Figure 3). These values were similar or even higher to those found in other studies of protein recovery from slurry-fed microalgae using different technologies, such as sonication and alkaline treatment at pH 12 [51], alkaline hydrolysis at pH 12 and acid precipitation [52]. However, the values were lower than those reported by Vanotti and Szogi [45] from spirulina algae, where an acid step (citric acid) at pH 3.1 was followed by a second alkaline extraction at pH 12.8, and that observed by Calvo-de Diego et al. [53] in a study of an integrated biorefinery approach to simultaneously recover nutrients, proteins and energy from pig manure.

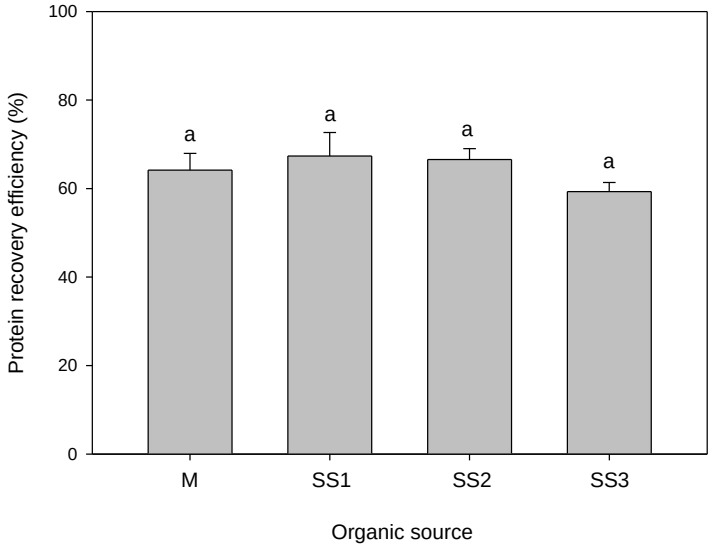


Figure 3. Protein recovery efficiency from the alkaline supernatants obtained with the optimized procedure. Vertical bars represent standard deviation. Mean values with the same letter are not significantly different at $p > 0.05$ (Tukey-b test).

3.5. Struvite Production: Chemical and Mineralogical Characterization of the Precipitates

Table 3 shows the chemical characterization of the struvite precipitates obtained from the acidic supernatants after applying the procedure for struvite precipitation. The precipitates from the sewage sludge samples (SS1, SS2 and SS3) had higher contents of total organic C, Ca, Na and Fe compared to the precipitate from the solid fraction of pig slurry (M). However, the rest of the elements were in higher concentrations in the precipitate from M, especially the basic elements of struvite (N, Mg and P), with only the precipitate from SS3 showing similar contents on these elements (Table 3). The low contents of N, P and Mg could have influenced the efficiency of struvite recovery method, since the process of struvite crystallization depends mainly on two operational factors, pH and the Mg:N:P molar ratio [54]. The pH must be alkaline, with optimal pH values generally between 8.5 and 9.5 [19], while the Mg:N:P molar ratio must be greater than 1:1:1. Shih et al. [55] established the Mg/N/P molar ratio of 1.3:4:1 as optimal, obtaining phosphorus recovery efficiencies of 95.8%. Thus, the limiting reagent is usually magnesium ion, which must be dosed [37,54]. Furthermore, as has been previously commented in the P recovery efficiency, the highest background concentration of iron in the samples SS1 and SS2 could strongly affect the efficiency of this recovery method, especially via struvite precipitation, since iron has a higher affinity for phosphate than magnesium and can bind phosphate, making it unavailable for its recovery as struvite [50,56]. In fact, Fe is used in the municipal wastewater treatment plants to prevent struvite formation [50]. This could partly explain the low recovery efficiencies observed in the samples SS1 and SS2, which showed the highest Fe concentrations. In addition, only the recovered precipitates from M and SS3 fulfilled the criterion of P_2O_5 contents (>16% of the dry matter content) established in the EU fertilizing products regulation [57], also verifying the criteria concerning heavy metal content (Table 3) [58].

Concerning the mineralogical characterization of the precipitates, the X-ray diffraction (XRD) and the field emission scanning electron microscope with a coupled energy dispersive X-ray system (FESEM-EDX) techniques were applied in all the precipitates except for those from SS1, due to their almost negligible N contents (<0.5%), which limited struvite formation.

The XRD analysis is the only qualitative method used to characterize the nature of struvite [59]. Thus, the results of the XRD analyses of the precipitates showed that only those from SS3 and M (Figure 4b,c) displayed a pattern (intensity and position of the diffraction peaks) that matched the pure struvite diffractogram (JCPDS 77-2303) [60]. This was also confirmed with the pattern of commercial struvite (Figure 4d), which indicates the formation of struvite crystals [61]. However, the precipitate from SS2 showed a completely different pattern, indicating that struvite was not formed in this sample. On the other hand, the FESEM-EDX technique provides surface characterization and identifies the principal components of the precipitates [59]. The FESEM-EDX analysis of the precipitates confirmed the results obtained with the XRD analysis (Figure 5).

The precipitates contained the basic elements of struvite (O, N, P and Mg) (EDX spectra), together with traces of other elements, which was consistent with struvite composition, except for the SS2 precipitate, which had low Mg content and did not yield struvite. These results have also been reported in previous works [59,61]. In addition, with the data obtained from the EDX spectra, a Rational Mineralogical Analysis (RMA) [48] was developed to estimate the proportion of the principal mineralogical phases, with the estimation considering only the presence of struvite and hydroxyapatite. This analysis is an estimative study based on the combination of quantitative chemical and qualitative mineralogical analyses, which allows the quantitative determination of the mineralogical phases of a sample [48]. The results of the RMA carried out in the precipitates, with confirmed the

presence of struvite (from M and SS3), showed estimated proportions of 94% of struvite and 6% of hydroxyapatite for the M precipitate, and of 83.2% of struvite and 16.8% of hydroxyapatite for the SS3 precipitate, also indicating the higher proportion of struvite in these precipitates. Furthermore, the FESEM images also revealed that the precipitates were mainly coarse (Figure 5c) and coffin-like (Figure 5e) struvite crystallization, respectively, in line with the findings by Xu et al. [59] in a study of phosphorus recovery from waste activated sludge.

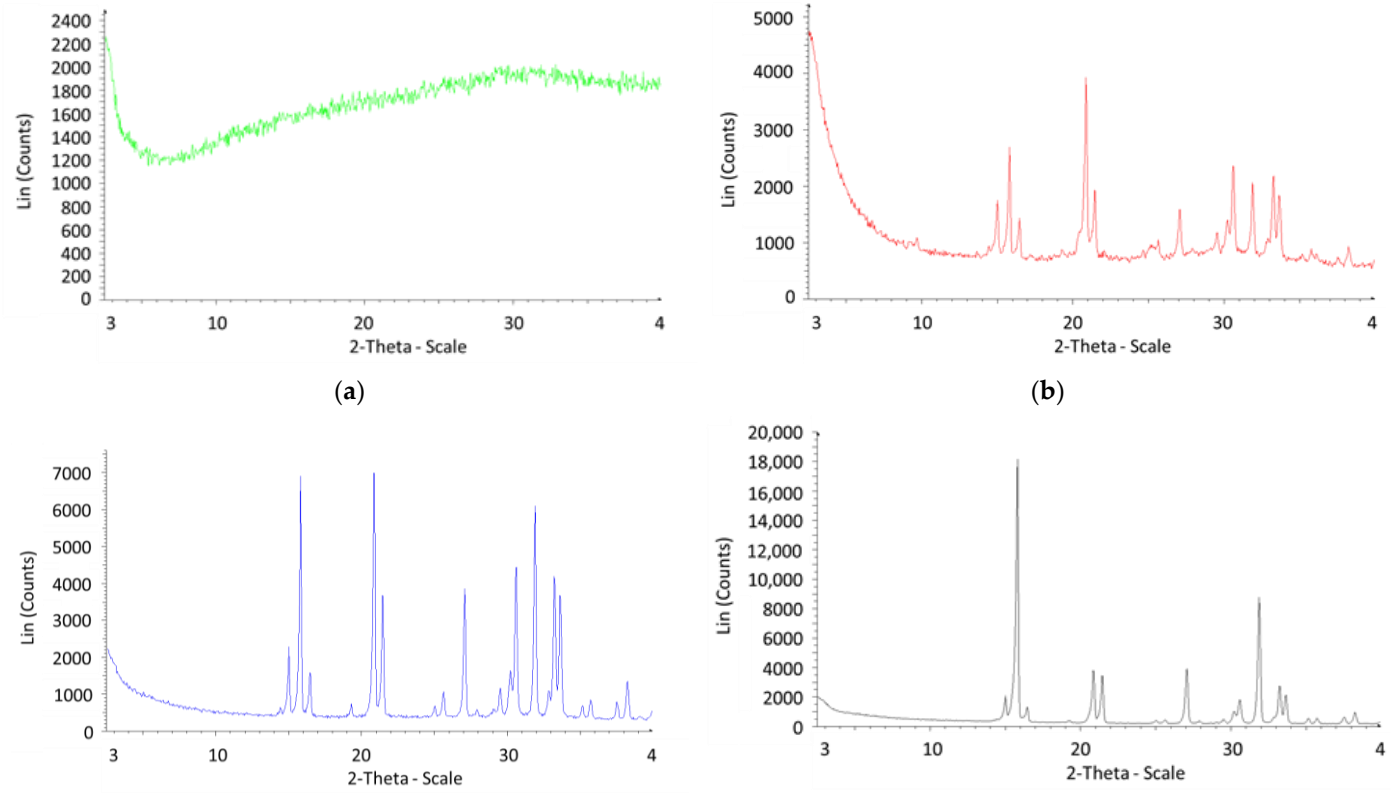


Figure 4. X-ray diffraction (XRD) patterns of the precipitates obtained: (**a**) precipitate from SS2 (sewage sludge from Castellón); (**b**) precipitate from SS3 (sewage sludge from Nules); (**c**) precipitate from M (solid fraction of pig slurry) and (**d**) sample of commercial struvite.

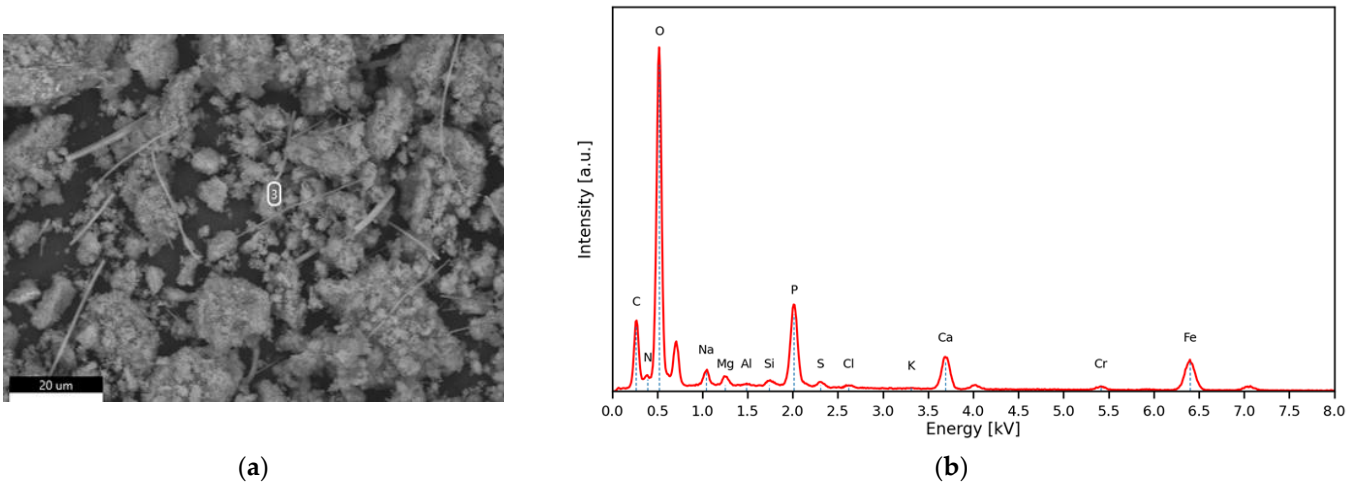


Figure 5. *Cont.*

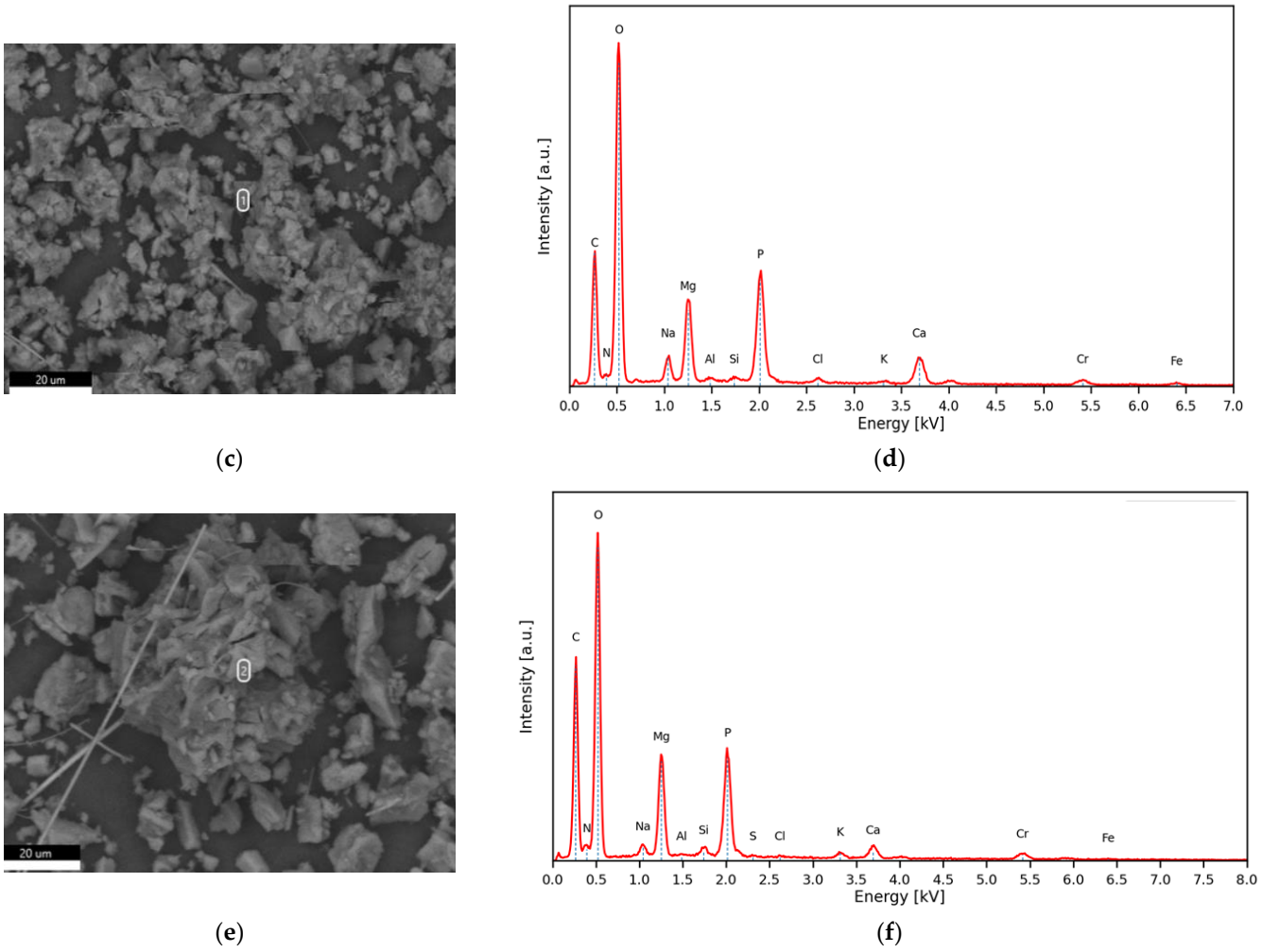


Figure 5. FESEM images with EDX of the precipitates obtained: (**a**,**b**) precipitate from SS2 (sewage sludge from Castellón); (**c**,**d**) precipitate from SS3 (sewage sludge from Nules); (**e**,**f**) precipitate from M (solid fraction of pig slurry).

4. Conclusions

The results obtained show that the phosphorus recovery process based on the modified methodology of Vanotti and Szogi [42], with an optimized bioacidification process through lactic fermentation using *Lactobacillus acidophilus*, constitutes a feasible alternative for phosphorus recovery from sewage sludge and pig slurry samples. The highest phosphorus recovery yields obtained were of 69% and 65.7%, for sewage sludge SS3 and the solid fraction of pig slurry (M), respectively. Moreover, the precipitates from the recovered P-rich supernatants of SS3 and M also showed the highest potential for struvite precipitation. These results show the strong influence of the organic waste characteristics on P recovery yield, especially the background Fe content of the organic sources used, which constitutes one of the main limitations for phosphorus recovery from sewage sludge samples via struvite precipitation. On the other hand, the efficiency of protein recovery through alkaline treatment of the remaining solid residues ranged from 59.3% to 67.4%, without showing a clear influence of the type of organic waste used. However, despite the promising results obtained, this study constitutes only an initial step for P recovery from sewage sludge using bioacidification processes. Thus, further research is necessary on process upscaling and operational factors, as well as on its economic cost, to confirm the viability of the process to obtain a market-ready product.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15102305/s1, Table S1: Studies on phosphorus recovery as struvite from different organic sources in the last 15 years.

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Abbreviations

The following abbreviations are used in this manuscript:

FESEM	Field emission scanning electron microscope
EDX	Energy dispersive X-ray system
XRD	X-ray diffraction
SS	Sewage sludge
M	Solid fraction of pig manure
PR	Phosphate rock
N	Nitrogen
P	Phosphorus

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